efficient transduction of skeletal muscles. Duchenne Muscular Dystrophy (DMD) is an example of a devastating muscle disorder without strongly effective treatment, which could benefit from the reconstitution of a deficient protein after rAAV-mediated gene transfer. DMD is a X-linked inherited muscle-wasting disease primarily affecting young boys with a prevalence of 1:5,000. The disease is caused by loss-of-function mutations in the gene encoding for the Dystrophin protein and is characterized by systemic, progressive, irreversible and severe loss of muscle function. Using a large network of laboratories with complementary skills, we are developing two rAAV-based gene therapy strategies for DMD. The first one stands on the constitutive expression of antisense oligonucleotides to promote, in the injected muscles, correction of the dystrophin messenger by exon skipping. The second one is based on the constitutive expression of a cDNA coding for a microDystrophin (µDys) protein. For our exon skipping approach, we use a gene therapy product consisting in a rAAV vector from serotype 8 (rAAV2/8) carrying a modified U7snRNA sequence promoting exon skipping to restore a shorter albeit functional quasi-dystrophin transcript. After having determined the therapeutic dose, the precise injection protocol and the toxicological/biodistribution patterns of this product in exhaustive pre-clinical studies, we are now in the phase of preparation of a Phase I/II clinical trial. This clinical trial will consist in the locoregional injection of the therapeutic rAAV2/8-U7snRNA vector in one forelimb of non- ambulant DMD patients. In parallel, our conventional gene-therapy approach is focused on the evaluation of a rAAV vector encoding a µDystrophin protein, for the treatment of DMD patients, whatever their genetic status. Using this vector, we injected a total of 12 Golden Retriever Muscular Dystrophy (GRMD) dogs, the canine model of DMD. We recently demonstrated that single-dose intravascular delivery of rAAV2/8-Spc512-µDys, in absence of immunosuppression, led to long-term transduction of distant muscle groups and extended lifespan (up to 2 years). Profound improvement of multiple clinical features was observed, including gait and respiratory parameters, and no toxicity or deleterious humoral and/or cell-mediated immune responses were observed.
The recent results and the specific developments of these translational projects will be presented.

Symposium- Parallel Symposium LGMD
• *T. Brand (UK)* • *Volker Straub (UK)* • *Isabelle Richard (FRANCE)*

A mutation in the cAMP-binding domain of *POPDC1* is causing a limb-girdle muscular dystrophy and cardiac arrhythmia
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The Popeye domain-containing (POPDC) genes encode a novel class of cAMP effector proteins, which is abundantly expressed in muscle and heart. In animal models (zebrafish and mouse), *Popdc1* and *Popdc2* are essential regulators of the structure and function of cardiac and skeletal muscle. However, until now, mutations in *POPDC1* or any other POPDC gene have never been associated with cardiac and skeletal muscle disease in patients. We recently identified a homozygous missense variant (c.602C>T, p.S201F) in POPDC1 by whole-exome sequencing in a family with cardiac arrhythmia and limb-girdle muscular dystrophy (LGMD). This allele was absent in known databases and segregated with the pathological phenotype in this family. The POPDC1^{S201F} allele was not found in a further screen of 104 patients with a similar phenotype, suggesting this mutation to be very rare. Serine 201 is located to the cyclic nucleotide-binding cassette in POPDC1 and a reduction in cyclic nucleotide binding was therefore predicted. Compared with WT protein, POPDC1^{S201F} mutant protein displayed a 50% reduction in cAMP affinity. Significantly, the mutant protein displayed a significantly altered subcellular localisation. In skeletal muscle from patients, the mutant POPDC1^{S201F} mutant protein as well as POPDC2 displayed impaired membrane trafficking and an enhanced perinuclear localisation was observed. Aberrant membrane trafficking and gating was observed when the mutant protein was co-expressed with TREK-1, a two-pore domain potassium channel in *Xenopus* oocytes. Forced expression of POPDC1^{S201F} in a murine cardiac muscle cell line (HL-1) increased hyperpolarization and upstroke velocity of the action potential. In zebrafish, expression of the homologous mutation (popdc1^{S191F}) caused heart and skeletal muscle phenotypes that resembled those observed in patients. Our study therefore identifies *POPDC1* as a novel and very rare autosomal recessive LGMD disease gene causing a mild muscular dystrophy and a severe AV-block by affecting protein trafficking and ion channel gating.
Studies in the author's laboratory were funded by the Medical Research Council (MR/J010383/1), the British Heart Foundation (PG/14/46/30911, PG/14/83/31128), and the Magdi Yacoub Institute.

Trial readiness for patients with limb girdle muscular dystrophy
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The limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of rare autosomal recessive and dominant diseases that clinically present with progressive weakness and wasting of shoulder and pelvic-girdle muscles. Over the last 20 years, the underlying genetic defects for many of the LGMDs have been identified and insight into pathomechanisms has been gained. Since we have entered an era of translational research for some of the more common forms of LGMD, the need for precise molecular diagnoses, a thorough understanding of the natural history of the diseases and guidelines for standardized assessments of the patients become even more relevant. There are a number of specific challenges at every stage of the translational research pathway. They include the incomplete knowledge and understanding of disease prevalence and disease course, genotype-phenotype correlation, modifying factors, as well as difficulties in identifying patients and accessing patient biomaterials. Moreover, traditional trial designs may be inappropriate or simply not feasible, the tools to measure clinical response to therapy may be lacking, and the relative costs of development and marketing are high. Next Generation Sequencing (NGS) and other Omics technologies, in combination with powerful IT infrastructure and sophisticated bioinformatics tools, now allow the deciphering of the entire genetic (exome, genome) profile of any LGMD patient, and the integration of this information with clinical data (deep phenotyping, ontologies, electronic health records), as well as data from natural history studies, interventional trials and biomarker studies. In this context, our team has started a project called MYO-SEQ, that focuses on the application of NGS, in particular whole exome sequencing (WES), in a large cohort of patients with unexplained limb-girdle weakness. Focusing on undiagnosed patients with a clearly defined clinical

phenotype enables increased diagnostic rates for known genes in this cohort, while the use of WES provides scope both for new gene discovery and for additional research into disease modifiers and genotype-phenotype correlation with substantial cost effectiveness. The MYO-SEQ project is exploring 1000 exomes of patients that were aged 10 years and above and presented with unexplained limb girdle weakness and an elevated serum CK activity. Patients were recruited from more than 50 sites across Europe. Results from the project will be presented.

AAV-mediated transfer of FKRP shows therapeutic efficacy in a murine model of limb-girdle muscular dystrophy type 2i, but requires tight control of gene expression

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Limb Girdle Muscular Dystrophies (LGMD) type 2I, a recessive autosomal muscular dystrophy, is caused by mutations in the Fukutin Related Protein (FKRP) gene. It has been proposed that FKRP, whose function remains unclear, is a participant in α-dystroglycan (αDG) glycosylation, which is important to ensure the cell/matrix anchor of muscle fibers. A knock-in mouse model of LGMD2I was generated to express the most frequent mutation (L276I) encountered in patients. The introduction of the mutation did not alter the expression of FKRP, neither at transcriptional nor at translational levels, but did alter its function since abnormal glycosylation of αDG was observed. In this model, skeletal muscles were functionally impaired from 2 months of age and a moderate dystrophic pattern was evident by histology starting from 6 months of age. Gene transfer with a rAAV2/9 vector expressing *Fkrp* restored the biochemical defects, corrected the histological abnormalities and improved the resistance to eccentric stress in the mouse model was obtained. However, injection of high doses of the vector induced a decrease of αDG glycosylation and laminin binding. Finally, we showed that intravenous injection of the rAAV-*Fkrp* vector into a dystrophic mouse model suffering of dystroglycanopathy due to skeletal muscle-specific Fukutin (*Fktn*) knock-out caused toxicity. The dose-dependent worsening of the dystrophic phenotype suggests requirement for a precise control of its expression.

Symposium- Parallel Symposium Myotonic Dystrophies
• *Bernard JASMIN (CANADA)* • *Denis Furling (FRANCE)* • *Guillaume Bassez (FRANCE)*

Staufen1 Acts as a Disease Modifier in DM1.

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For several years, we have been interested in studying the molecular mechanisms that control expression of genes encoding synaptic proteins in both muscle and nerves. Although initially, the emphasis of this work was on elucidation of transcriptional events, we have become increasingly interested in studying post-transcriptional mechanisms. As part of our efforts to identify RNA-binding proteins that play a key role in skeletal muscle, we became interested in Staufen several years ago. Staufen is a RNA-binding protein that associates with RNA secondary structures, primarily through one or more double-stranded RNA-binding domains. The role of Staufen is perhaps best characterized in Drosophila, where it functions in the transport and localization of distinct mRNAs in oocytes and embryonic neuroblasts. Studies in mammals revealed that there are two genes, Staufen1 and Staufen2, and that Staufen1 also regulates mRNA stability in a mechanism referred to as Staufen1-mediated mRNA decay (SMD), as well as translation of a subpopulation of transcripts when bound to the 5'UTR.
Our studies focusing on the role of Staufen1 in skeletal muscle has shown that Staufen1 accumulates at the level of the post-synaptic membrane of the neuromuscular junction where its expression varies according to the state of differentiation and innervation of muscle cells, while being also influenced by the presence of agrin and heregulin (Bélanger et al., 2003). Our more recent work revealed that expression of Staufen1 is markedly increased in muscle samples from DM1 patients and DM1 mouse models, and that it can regulate alternative splicing of pre-mRNAs including the insulin receptor and chloride channel, while also promoting the nuclear-cytoplasmic transport of mutant CUG^{exp} DMPK mRNAs (Ravel-Chapuis et al., 2012). High-throughput RT-PCR assays with DM1 myoblasts further showed that Staufen1, in fact, has a broad impact on several alternative splicing events, with some events predicted to be beneficial for DM1 and others not (Bondy-Chorney et al., 2016). Moreover, we observed that Staufen1 levels decrease during embryonic muscle development. Sustained expression of Staufen1 negatively affects myogenic differentiation, independent of SMD, by controlling translation of c-myc (Ravel-Chapuis et al., 2014). Finally, we have also observed that Staufen1 is recruited into stress granules in normal myoblasts and myotubes. In contrast, DM1 myoblasts formed such granules much less efficiently in response to stress in a Staufen1-dependent and cell-specific manner (Ravel-Chapuis et al., 2016). Collectively, our findings show that Staufen1 is a novel splicing regulator that assumes multiple additional functions in normal and DM1 muscle cells thereby indicating its role as a disease modifier in DM1.
This work is supported by the AFM, MDA (USA), CIHR (Canada), and Muscular Dystrophy Canada.

Neutralize RNA toxicity induced by expanded-CUG repeats in Myotonic Dystrophy

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Myotonic Dystrophy type 1 (DM1), one of the most common form of inherited neuromuscular disorders in adults is characterized by myotonia, progressive muscle weakness and wasting, cardiac defects, endocrine troubles and cognitive impairments. This autosomal dominant disease is caused by an expanded tract of trinucleotide (CTG)n>50 repeats located in the 3' non-coding region of the *DMPK* gene. The size of the expansion is generally correlated with the clinical severity and the age of onset of the disease. Expression of pathogenic *DMPK* transcripts containing expanded CUG repeats (CUGexp-RNAs) results in a toxic RNA gain-of-function mechanism. CUGexp-RNAs are retained into the nucleus as riboprotein